Attach To Paper
NO # 10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
HALLENBECK *et al.*	Art Unit: 1633
Appl. No.: 08/849,117	Examiner: Nguyen, D.
Filed: August 1, 1997	Atty Docket: 1136.0020002/RWE/BJD
For: **Vectors for Tissue-Specific Replication**	

Considered on 12/29/98

Declaration Under 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

The undersigned, Paul L. Hallenbeck, Ph.D., declares and states that:

1. I am an inventor of the above-captioned U.S. patent application number 08/849,117, filed August 1, 1997, entitled "Vectors for Tissue-Specific Replication."

2. I am employed as a Program Head with Genetic Therapy, Inc., in Gaithersburg, Maryland, the assignee of record of the above-referenced patent application.

3. I am the subject of the *Curriculum Vitae* attached hereto, and I am the author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents, I submit that I am an expert in the field of gene therapy for cancer, which includes being skilled in the arts of molecular biology, virology, oncology and cell biology.

I have been a speaker at numerous international scientific meetings in these fields, and I am qualified to speak on the skill and knowledge of those of ordinary skill in these fields.

4. I have read and understand the subject-matter of the above-captioned patent application.

5. I have read and understand the Office Action dated April 13, 1998, particularly the sections at pages 4-9 in which claims 1-40 have been rejected under 35 U.S.C. § 112, first paragraph, for lack of sufficient written description and lack of an enabling disclosure.

6. I respectfully disagree with the contentions in the Office Action, and state that the present specification reasonably conveys to one of ordinary skill in the art that my co-inventors and I had possession of the subject matter of claims 1-40, and enables one of ordinary skill in the art to make and use the subject matter of claims 1-40. These statements are based on: (a) the results demonstrated in the specification as filed, particularly in Examples 1 and 2, and Figures 2 and 3; (b) results from additional experiments conducted in the laboratories of Genetic Therapy, Inc., by me or by personnel of Genetic Therapy, Inc., under my supervision; and (c) results reported in the literature in which the methods described in the present specification were used.

A. Results in the Specification

7. Example 1 of the present specification (see pages 32-35) demonstrates the construction of an adenovirus vector (AVAFPE1a), comprising the hepatoma-specific α-fetoprotein (AFP) promoter operatively linked to the adenoviral E1a gene. The AVAFPE1a

vector is the same as the AVE1a04i vector which is described elsewhere in the specification, and which is referred to below in this Declaration and the accompanying Figures. In Example 1, at pages 35-36 and in Figure 3, the specification demonstrates that this vector replicates and produces cytopathic effects specifically in HuH7 cells which express AFP, but not in A549 cells which do not express AFP. Hence, the AVAFPE1a (AVE1a04i) construct is an example of a vector comprising a heterologous tissue-specific regulatory sequence (in this example, the AFP promoter) that is specifically activated for replication only in AFP-expressing target tissues (such as HuH7 hepatoma cells, as in this example).

B. Results from Additional Experiments

8. Since the filing of the present application, additional experiments have been conducted in the laboratories of Genetic Therapy, Inc., by me or by personnel of Genetic Therapy, Inc., under my supervision. These experiments were designed to survey the ability of vector constructs to replicate and/or induce cytopathic effects (CPE) in broad array of AFP-expressing (hereinafter "AFP$^+$") and AFP-nonexpressing (hereinafter "AFP$^-$") cell lines. In initial experiments, a variety of cell lines and primary human cells were examined for their ability to express AFP. Cells used in these experiments included hepatoma cell lines (Hep3B, HepG2, HuH7 and HLF), non-hepatoma cell lines (AE1-2a (also known as A30), A549, LoVo, H460, HeLa and Chang), and primary human lung cells (NHBE and HMVEC-L). Cells were cultured *in vitro* for 10 days, after which the levels of AFP contained in culture supernatants were determined by ELISA using a commercial AFP ELISA system. As shown in Figure A attached hereto, the human hepatoma cell lines Hep3B, HepG2 and HuH7 expressed significant quantities of AFP, while non-hepatoma cell lines and primary human lung cells did not.

9. These cell lines were then transfected with one of several different vector constructs, to determine the ability of the vector constructs to replicate and/or induce CPE in the cell lines, and to demonstrate that vector constructs containing a gene essential for viral replication under control of the AFP gene could only replicate in cell lines expressing AFP. The above-noted cell lines and primary cells were transfected with one of the following: (a) a positive control vector construct (the replication-competent adenovirus construct Addl327, which resembles wildtype adenovirus in its ability to replicate constitutively); (b) a negative control vector construct (the replication-defective adenovirus construct Av1nBg01v, which is deficient in E1a production and therefore cannot replicate except in cells expressing E1a constitutively); and (c) a tissue-specific, replication-conditional adenoviral vector construct (AvE1a04i, *i.e.*, the same vector referred to above as AVAFPE1a, containing the E1a gene operably linked to the AFP promoter). As shown in Figure B attached hereto, the positive control Addl327 vector construct replicated and induced CPE in all of the cells tested. When these same cells and cell lines were transfected with the negative control Av1nBg01v, the virus only replicated in AE1-2a (A30) cells, which complement the E1a deficiency of the virus in *trans* (Figure C). Finally, as shown in Figure D attached hereto, when the cells were transfected with the AvE1a04i construct, the vector was able to replicate and induce CPE in the AFP^+ cell lines Hep3B and HepG2, and in the positive control AE1-2a cell line, but not in any of the AFP^- cell lines in which the E1a gene was not activated. These results were confirmed in the photomicrographs depicted in Figure E attached hereto: the Addl327 construct induced cytopathic effects in all cell lines tested regardless of their expression levels of AFP; the AVE1a04i construct replicated specifically in those cells expressing AFP (Hep3B) and in positive control cells expressing E1a in *trans* (A30, *i.e.*, the positive control cell line referred to above as AE1-2a), but not in those cells that did not express AFP (Chang);

and the replication-defective adenovirus Av1nBg01v (the same vector construct as that depicted in Figure E as AV1LacZ4) was only capable of replication in positive control cells (A30).

10. Analogous results were obtained with primary hepatocytes *in vitro*. In these studies, adult swine and human hepatocytes (which do not express AFP), and the fetal swine hepatocyte cell line PICM19 (which constitutively express AFP), were transfected with the positive control Addl327 vector or with the tissue-specific, replication-conditional adenoviral vector construct AvE1a04i. As shown in Figure F attached hereto, the positive control Addl327 vector replicated and induced CPE in all of the cells tested, regardless of their level of AFP expression. When cells were transfected with the AvE1a04i construct, however, replication and CPE were only observed in the AFP^+ PICM19 cell line, and not in adult swine or adult human hepatocytes that are AFP^-.

11. We have also conducted experiments designed to demonstrate the efficacy of the claimed tissue-specific, replication-conditional vectors in ablating tumors *ex vivo* and *in vivo*. In a first set of studies designed to simulate *ex vivo* treatment of tumor-containing tissue, AFP^+ tumor cells (Hep3B) or AFP^- cell lines (Chang) were pre-transfected *in vitro* with the AvE1a04i construct and then injected into nude mice. As shown in Figure G attached hereto, tumors developed only in those mice injected with pre-treated Chang cells (in which vector replication and cell killing do not take place), but not in mice injected with pre-treated Hep3B cells (which express AFP and therefore in which the AvE1a04i vector is able to replicate and kill the cell).

12. In related studies designed to demonstrate the ability of the vector constructs to ablate pre-established tumors *in vivo*, Hep3B cells were injected into nude mice and xenographic tumors established in the mice, and mice then treated with nothing ("untreated"), with injection vehicle only ("mock"), with positive control vector (Addl327), with negative control vectors (Av1nBg and Av3nBg), and with a tissue-specific, replication-conditional vector of the invention (AvE1a04i). As shown in Figure H attached hereto, mice that were untreated or only mock transfected all died with 56 days. Less than 40% of mice treated by injection with negative control vectors (Av1nBg and Av3nBg) survived longer than about 60 days, while about 80% of mice injected with the positive control vector (Addl327) survived for over 95 days. Injection of the AvE1a04i vector into tumor-bearing mice also provided significant enhancement of viability: after 98 days, over 50% of these mice remained alive. These results indicated that the AvE1a04i vector construct is able to localize to the tumor tissue in these mice, selectively replicate therein and ablate the tumors.

13. Together with those reported in the specification, these results demonstrate that the methods described in the present specification fully teach the production and use of vector constructs comprising a tissue-specific regulatory sequence (such as the AFP promoter) that are tissue-specific for replication.

C. Results Reported in the Literature

14. In further support of the enablement of the claimed invention by the present specification, there have now been several reports demonstrating the use of the present methods to successfully produce tissue-specific replication-conditional vectors that selectively kill target

cells *in vitro* and *in vivo*. For example, in Rodriguez *et al.*, *Cancer Res.* 57:2559-2563 (1997) (hereinafter "Rodriguez," a copy of which is attached hereto as Appendix A), the investigators constructed vectors comprising enhancer/promoter regions from the human prostate-specific antigen (PSA, produced by prostatic carcinoma cells but not by normal prostate tissue), operatively linked to the adenovirus E1a gene. These vectors were transfected into a variety of cell lines, and were found to specifically replicate in, and kill, those tumor cell lines expressing PSA but not those that did not express PSA. In addition, injection of the vectors into nude mice bearing prostate tumors resulted in complete elimination of the tumors, while injection into mice bearing tumors that did not express PSA did not significantly inhibit tumor growth or survival. Thus, as concluded in Rodriguez, vectors constructed and used in methods according to the present invention demonstrate "tumor cell selectivity and tumor cell killing . . . . " Rodriguez at page 2561, col. 1, fourth full paragraph, lines 1-2.

15. Analogous results have been obtained in the following additional reports:

Reference	Viral Construct Used	Appendix:
Chen *et al.*, *J. Clin. Invest.* 96:2775 (1995)	DF3-HSV-*tk*	B
Wills *et al.*, *Canc. Gene Therap.* 2:191 (1995)	CMV-HSV-*tk*; AFP-HSV-*tk*	C
Siders *et al.*, *Cancer Res.* 56:5648 (1996)	tyrosinase-β-galactosidase	D
Kanai *et al.*, *Cancer Res.* 57:461 (1997)	AFP-cytosine deaminase	E
Lan *et al.*, *Cancer Res.* 57:4279 (1997)	CEA-cytosine deaminase	F

These reports demonstrate that vector constructs comprising a toxic gene (Chen, Wills, Kanai and Lan) or a marker gene (Siders) under control of a heterologous tissue-specific promoter will transfect, specifically replicate in, and kill or express the marker in, those tumor cell lines

expressing the respective tissue-specific factor (*i.e.*, DF3 in Chen; AFP in Wills and Kanai; tyrosinase in Siders; and CEA in Lan), but not those cells that did not express the tissue-specific factor.

16. The vectors used in the above-referenced reports are analogous to those of the present invention, wherein a gene essential for viral replication is operatively linked to a heterologous tissue-specific transcriptional regulatory sequence. Just as the vectors in the above-referenced report functioned to facilitate transcription of the toxic or marker gene in the transfected cells, under control of a heterologous promoter, the presently claimed vectors function to facilitate transcription of a gene essential for viral replication (and thus viral replication) under control of a heterologous transcriptional regulatory sequence such as a tissue-specific promoter. In addition, a variety of tissue-specific transcriptional regulatory sequences that may be used in the claimed vector constructs and methods are now known in the art (*see, e.g.*, Tables 1 and 2 at cols. 1-15 of U.S. Patent No. 5,728,379 to Martuza *et al.*, which was made of record by the Examiner in the present Office Action but which is post-filing art to the present application, as noted at page 36 in Applicants' Amendment and Reply Under 37 C.F.R. § 1.111 filed herewith). Since the above-referenced reports demonstrate expression of a non-cellular gene under control of a heterologous transcriptional regulatory sequence, and since a variety of tissue-specific transcriptional regulatory sequences are now known in the art, one of ordinary skill in the art would fully expect, and would have no reason to doubt, that the presently claimed vector constructs would work in the selective ablation of cells (particularly tumor cells) *in vitro*, *ex vivo*, and *in vivo*, by carrying out the claimed methods as fully described in the present specification.

17. Thus, it is clear from (a) the results presented in the present specification; (b) the results of additional studies performed in our laboratories; and (c) the results available in the post-filing art, that the claimed tissue-specific, replication-conditional vectors, and the isolated cells containing and methods using such vectors, can be produced using the methods described in the present specification without undue experimentation.

18. I have read, I am familiar with, and I understand, the provisions of 37 C.F.R. §§ 10.18(b) and (c) relating to the effect of signature and certificate for correspondence filed in the U.S. Patent and Trademark Office.

October 13, 1998
Date

Paul L. Hallenbeck, Ph.D.

CURRICULUM VITAE

PAUL LEON HALLENBECK

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Personal Information:

Date of Birth - 4/03/59
Place of Birth - Schenectady, NY
Married/4 year old daughter/1 year old son.

EDUCATION:

Ph.D. in Microbiology, University of Illinois, Urbana, IL (1984-1989). Title of Dissertation: "Identification and Regulation of Carbon Fixation Genes in Rhodobacter sphaeroides." **GPA-4.8/5.0**

M.S. in Microbiology, University of Illinois, Urbana, IL (1982-1984). Title of Preliminary Examination paper: "Isolation of the Form I D-Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Gene and the Preliminary Characterization of the Form II Gene from Rhodopseudomonas sphaeroides."

B.S. in Chemistry, Magna cum laude, Siena College, Loudonville, NY (1982). **GPA-3.65/4.0**

A.A.S. in Medical Laboratory Technology, Hudson Valley Community College, Troy, NY (1979).

PROFESSIONAL EXPERIENCE:

Program Head-Tumor Targeting, Dr. Forry-Schaudies (Unit Head-Oncology (1997-present).

Scientist III, Dr. Yawen Chiang (Vice President of Preclinical Studies, Immunology, and Animal Studies), and Dr. Yung-Nien Chang (Group Leader-Gene Expression)

Genetic Therapy, Inc. (1996-1997).

Scientist II, Dr. Yawen Chiang (Vice President of Preclinical Studies, Immunology, and Animal Studies), and Dr. Yung-Nien Chang (Group Leader-Gene Expression) Genetic Therapy, Inc. (1993-1996).

Achievements/Responsibilities

- Team leader for development and testing of adenoviral vectors for gene therapy of cancer. Directly responsible for the
  - Design, construction, purification, production of adenoviral vectors specific for gene therapy of cancer.
  - Planning, execution, and evaluation of experiments with tumor cell lines and primary culture designed to demonstrate functional efficacy and specificity *in vitro*.
  - Planning, execution, and evaluation of experiments with syngeneic and immunodeficient animal tumor models designed to demonstrate functional efficacy and specificity *in vivo*.
  - Planning, execution, and evaluation of safety studies with these vectors.
  - Initiation, development and testing of a mouse model to determine adenoviral immune responses.
  - Presentation and reporting of activities associated with these projects.
  - Initiating and evaluating potential collaborators and pursuing assistance from service companies.
- Animal Tumor Modeling
  - syngeneic and immunodeficient mice and rat models of various human cancers
  - evaluation and initiation of models outside GTI
- Reviews (grants, manuscripts, patents, research proposals, collaborations, in house data, etc)
- Presentations (management, the immunology department, visiting scientists, outside collaborators, company wide, national and international meetings).
- Write manuscripts, preclinical reports, project reviews, RBM reports, and various other reports.
- Work with collaborators-
- Initiate novel patentable ideas
- Principle investigator on 6 animal care and use protocols.
- Principle investigator on GLP preclinical safety study of an adenoviral vector for gene therapy of cancer.
- Wrote and approval of 4 IBC protocols.
- Assisted in all phases of retroviral-tk treatment of hepatocellular carcinoma project.
- Supervisor (5-15 FTE)

Staff Fellow, Dr. Vera Nikodem (Senior tenured scientist), National Institutes of Health. (1991-1993)

National Research Council Fellow, Dr. Vera Nikodem (Senior tenured scientist), National Institutes of Health. (1989 to 1991)

Achievements

- Demonstrated heterodimerization between thyroid hormone receptor and RXRβ, the receptor for 9-*cis* retinoic acid. Utilized baculovirus/Sf9, E. coli, and *in vitro*-transcription/translation expression systems, DNA and ligand binding assays, cross-linking, immunological detection schemes, protein purification, post-translational modification assays such as phosphorylation, and a variety of transient and stable transfections in different tissue culture cell-lines.
- Discovered that heterodimerization led to enhanced binding of each member of the heterodimer to several specific DNA sequences found within genes regulated by thyroid hormone or retinoic acid and that the transcriptional activity of each was either dramatically enhanced or inhibited by heterodimerization with the other partner. Depended upon the specific combination(s) of DNA sequence, structure, subtype of receptor, and ligand.
- Identified novel functions of the domains of these receptors through the construction and testing of PCR derived chimeras between the different isoforms. Information may yield clues into the understanding how these ligands and receptors function in controlling certain cascades of normal gene regulation, such as those involved in hematopoiesis, differentiation, development and abnormal gene regulation such as in cancer.

Research Assistant and dissertation studies synopsis, Dr. Samuel Kaplan, University of Illinois, Department of Microbiology. (1982 to 1989)

Achievements

- Identified, cloned, and characterized several of the genes of the Calvin cycle in the photosynthetic bacterium Rhodobacter sphaeroides. One region of the R. sphaeroides chromosome was found to contain the genes coding for fructose 1,6-bisphosphatase(fbpA), phosphoribulokinase(prkA), a 37 kDa polypeptide(cfxA), and form I ribulose 1,5-bisphosphate carboxylase/oxygenase (rbc L,S). These genes were found to be tandemly arranged, and expressed from separate operons in the same transcriptional direction. A second, unlinked region of the other R. sphaeroides chromosome was found to contain a duplicate (with respect to functionality of the gene products) but not identical set of these same four genes.

Utilized molecular biological, biochemical, immunological techniques, as well as bacterial genetics .

- First report on the cloning of the gene coding for phosphoribulokinase, a key and unique enzyme in the Calvin cycle from any source.
- Demonstrated redox transcriptional control for the Calvin cycle by performing site directed mutagenesis of each individual prk and cfx gene as well as both prk genes together and both cfx genes together.

Teaching Assistant, Dr. Charles Pratt, University of Illinois, Department of Microbiology. Taught general microbiology laboratory and lecture for medical students and microbiology majors. (1982 to 1984)

Summer Internship, Dr. Robert Fraley, Monsanto Corporation, St. Louis, MO. Analytical identification and quantification of the plant metabolites auxin and cytokinin in transformed Agrobacterium tumefaciens and petunia strains. Applied molecular biological techniques in the analysis of those strains. (Summer, 1982).

Research Technician, Sterling Winthrop Research Institute, Troy NY. Quality control and test validation. (1979-1980)

HONORS/PROFESSIONAL ORGANIZATIONS:

New York Academy of Sciences Membership (submitted upon request)
National Research Council Research Associate Award, 1989-1991
Invitation to Who's Who in Science and Engineering
American Society for Microbiology
American Association for the Advancement of Science
Department of Health and Human Services, **Public Health Service**
Training Grant Award, 1984-1987
Excellence in Major Field from the American Institute of Chemistry, Siena College
National Physics Honor Society, (Sigma Pi Sigma, Siena College)
Beta Beta Beta Biological Society (Chi Alpha, Russell Sage College, Troy, NY)
Elected to Congregational Council (St. Luke Lutheran Church, 2500 members)

PATENTS:

1. **Hallenbeck, P.L.**, Chang, Y-N., Chiang, Y.L. Vectors for Tissue Specific Replication. Filed. 1994. Sponsor-Genetic Therapy, Inc. U.S. Patent Application No. 08/348,258 and 08/487,992, Foreign filed 1995.

2. **Hallenbeck, P.L.**, Ramsey J.W., Chiang Y.L., Hammer M., Tissue-Specific Treatment, Diagnostic Methods, and Compositions Using Replication-Deficient Vectors. Filed. 1994. Sponsor-Genetic Therapy, Inc. U.S. Patent Application No. 08/468,815. Foreign filed

1995.

3. Chiang, Y.L., **Hallenbeck, P.L.**, McGarrity G., and Anderson F., Gene Therapy of Hepatocellular Carcinoma Through Cancer-Specific Gene Expression. U.S. Patent Application No. 08/444,284. Foreign filed 1996.

4. Chang, Y-N, Chiang, Y.L., **Hallenbeck, P.L.**, Novel SV40 Vector for Gene Therapy. Invention disclosure. June, 1996.

PUBLICATIONS:

1. **Hallenbeck P.L.,** Minucci S., Lippoldt R., Phyillaier M., Horn V., Ozato K., and V. Nikodem. Differential 9-*Cis* Retinoic Acid-Dependent Transcriptional Activation by RXR α and RXR β: Role of Cell Type and RXR Receptor Domains. **J. Biol. Chem.** 271:10503-10507, 1996

2. **Hallenbeck P.L.**, Ramsey W.J., Golightly D., Hammer M., Gordon E.M., Chang Y-N., Anderson F., McGarrity G., Blaese M., and Y. Chang. Replication of E1 Deleted Adenoviral Gene Therapy Vectors in Tumor Cell Lines. Prepared for submission to Nature Medicine, 1997.

3. **Hallenbeck P.L.**, Hay C., Golightly D., Chang Y-N., McGarrity G., and Y. Chang. A Novel Tumor Specific Replication Competent Adenoviral Vector for Gene Therapy of Hepatocellular Carcinoma. Prepared for submission to Human Gene Therapy. 1997.

(3 more in preparation with J. Ramsey/M. Blaese at NIH)

4. Kaneko, S., **Hallenbeck, P.L.**, Kotani, T., Nakabayashi H., McGarrity G., Tamaoki T., Anderson W.R., and Y.L. Chiang. Adenovirus-mediated Gene Therapy of Hepatocellular Carcinoma Using Cancer-specific Gene Expression. **Cancer Res.** 55:5283-5287, 1995.

5. **Hallenbeck, P. L.**, Phyillaier M., and V. M. Nikodem. Divergent effects of RXRβ on positive and negative thyroid hormone receptor dependent gene expression. **J. Biol. Chem**. 268:3825-3828, 1993.

6. Khoury, A. M., King D. L., Hatzivassiliou E., Casas, L. E., **Hallenbeck P. L.**, Nikodem V. M., Mitsialis S.A., and Kafatos F.C. DNA binding and heterodimerization of the Drosophila transcription factor CF1/USP. **PNAS** 89:11503-11507, 1992.

7. **Hallenbeck, P. L.**, Marks, M. S., Lippoldt, R. E., Ozato, K., and Nikodem, V. Heterodimerization of thyroid hormone receptor with H-2RIIBP (RXRβ) enhances DNA binding and thyroid hormone dependent transcriptional activation. **PNAS** 89:5572-5576,

1992.

8. Marks, M. S., **Hallenbeck, P. L.**, Nagata, T., Segars, J. H., Appella, E., Nikodem, V., and Ozato, K. H-2RIIBP (RXRβ) heterodimerization provides a mechanism for combinatorial diversity in the regulation of retinoic acid and thyroid hormone responsive genes. **EMBO** 11:1419-1435, 1992.

9. Farsetti, A., Desvergne, B., **Hallenbeck, P.**, Robbins, J., and Nikodem, V. Characterization of myelin basic protein thyroid hormone response element and its function in the context of native and heterologous promoters. **J. Biol. Chem.** 267:15784-15788, 1992.

10. **Hallenbeck, P. L.**, Lerchen, R., Hessler, P. and Kaplan, S. Roles of CfxA, CfxB, and external electron acceptors in regulation of ribulose 1,5-Bisphosphate carboxylase/oxygenase expression in Rhodobacter sphaeroides. **J. Bacteriol.** 172:1736-1748, 1990.

11. **Hallenbeck, P. L.**, Lerchen, R., Hessler, P. and Kaplan, S. Phosphoribulokinase activity and regulation of CO_2 fixation critical for photosynthetic growth of Rhodobacter sphaeroides. **J. Bacteriol.** 172:1749-1761, 1990.

12. **Hallenbeck, P. L.** Identification and regulation of genes involved in carbon dioxide fixation in Rhodobacter sphaeroides. **Ph.D. Thesis, 1-225**, 1989.

13. **Hallenbeck, P. L.** and Kaplan, S. Structural gene regions of Rhodobacter sphaeroides involved in CO_2 fixation. **Photosynthesis Research** 19:63-71, 1988.

14. **Hallenbeck, P. L.** and Kaplan, S. Cloning of the gene for phosphoribulokinase activity from Rhodobacter sphaeroides and its expression in Escherichia coli. **J. Bacteriol.** 169:3669-3678, 1987.

REFEREES:

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furnished upon request

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SELECTED SEMINARS:

1. **Hallenbeck, P. L.**, and V. Nikodem. "Heterodimerization of nuclear hormone receptors and its functional significance" NIH research symposium, Bethesda, MD, 1992.

2. **Hallenbeck, P. L.**, Marks, M., Mitsuhashi, T., Ozato, K., and V. Nikodem. "Alterations in functional and binding properties of the thyroid hormone receptor by heterodimerization with a novel member of the steroid hormone receptor superfamily." American Thyroid Association Meeting, Boston, MA, 1991.

3. **Hallenbeck, P. L.** and Kaplan, S. "Mutational analysis of two structural gene regions of Rhodobacter sphaeroides involved in CO_2 fixation." VI Symposium of Phototrophic prokaryotes, Noordwijkerhout, Netherlands, August, 1988.

4. **Hallenbeck, P. L.** "Differences in expression of PRK activity of the two prk genes from Rhodobacter sphaeroides." Department of Microbiology, Annual Allerton Conference, University of Illinois, Urbana, IL, 1988.

5. **Hallenbeck, P. L.** "Expression of phosphoribulokinase in Rhodobacter sphaeroides." Department of Microbiology, Clemson University, Clemson, SC, 1987.

SELECTED ABSTRACTS:

1. **Hallenbeck, P. L.**, Mitsuhashi, T., and Petty, K. J. " Binding of thyroid hormone receptor to a thyroid hormone response element of malic enzyme gene is non-competitively inhibited by a variant of the thyroid hormone receptor which does not bind thyroid hormone." Annual Meeting of the Endocrine Society, Atlanta, GA, 1990.

2. **Hallenbeck, P. L.** and Kaplan, S. "Construction and characterization of a mutation in prka from Rhodobacter sphaeroides." Molecular Biology of Photosynthetic Procaryotes Meeting, University of Wisconsin, Madison, WI, 1987.

3. **Hallenbeck, P. L.** and Kaplan, S. "Comparison of phosphoribulokinase from Rhodobacter sphaeroides and the R. sphaeroides gene product synthesized in Escherichia coli." Annual Meeting of the American Society for Microbiology, Atlanta, GA, 1987.

4. **Hallenbeck, P. L.** and Kaplan, S. "Cloning of the gene for phosphoribulokinase activity from Rhodopseudomonas sphaeroides and its expression in Escherichia coli." Annual Meeting for the American Society for Microbiology, Washington, D.C., 1986.

MANUSCRIPTS IN PREPARATION:

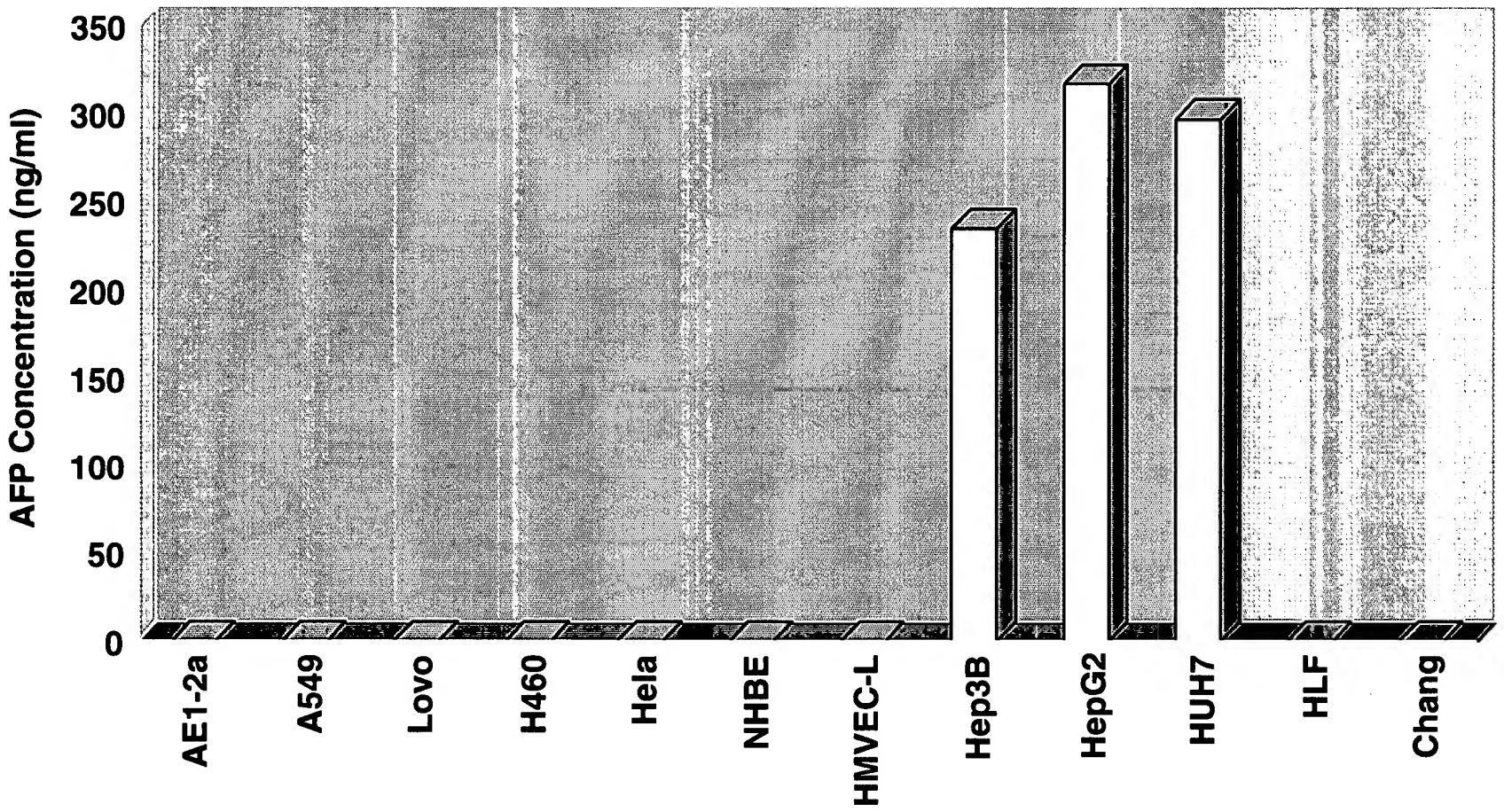
1. **Hallenbeck, P.L.**, Ramsey J.R., Blaese, M., and Y. Chiang. Replication of adenoviral gene therapy vectors in P53- tumor cells.

2. Ramsey J.R., **Hallenbeck, P.L.**,Chiang, Y., M. Blaese. Gene Therapy of glioma utilizing an adenovirus derived vector.

3. Kaneko, S., Matsushita, E., **Hallenbeck, P. L.**, Jonathan, D-J., Kotani T., W.F. Anderson, and Y. Chiang. Gene Therapy for the treatment of mouse hepatocellular carcinoma using intratumoral transduction with the herpes simplex thymidine kinase gene/ganciclovir system.

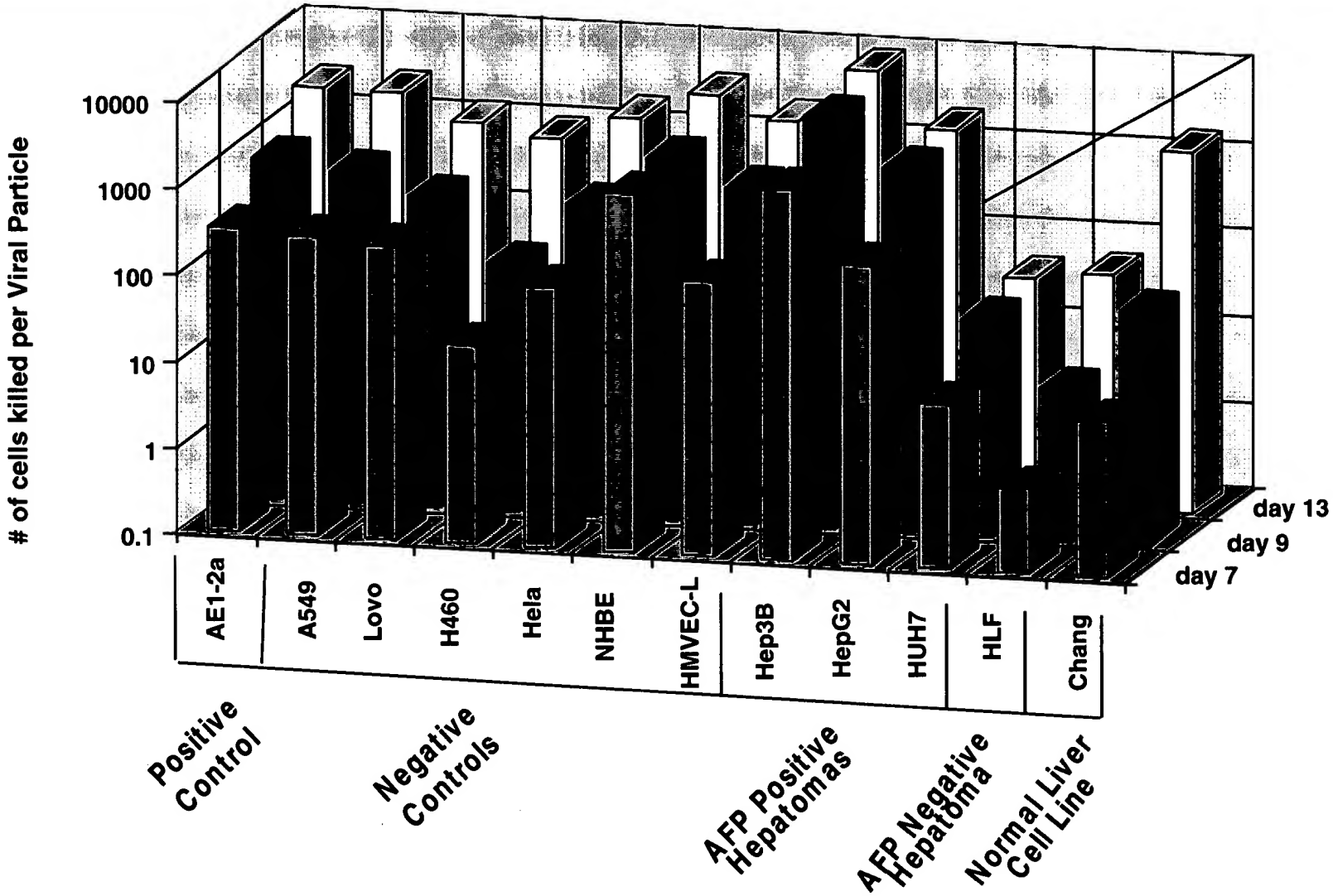
4. **Hallenbeck P. L.**, Lippoldt, R. E., Ozato, K. and V. M. Nikodem. Mechanism of RXR dependent control of thyroid hormone and retinoic acid receptor mediated gene expression.

AFP Expression



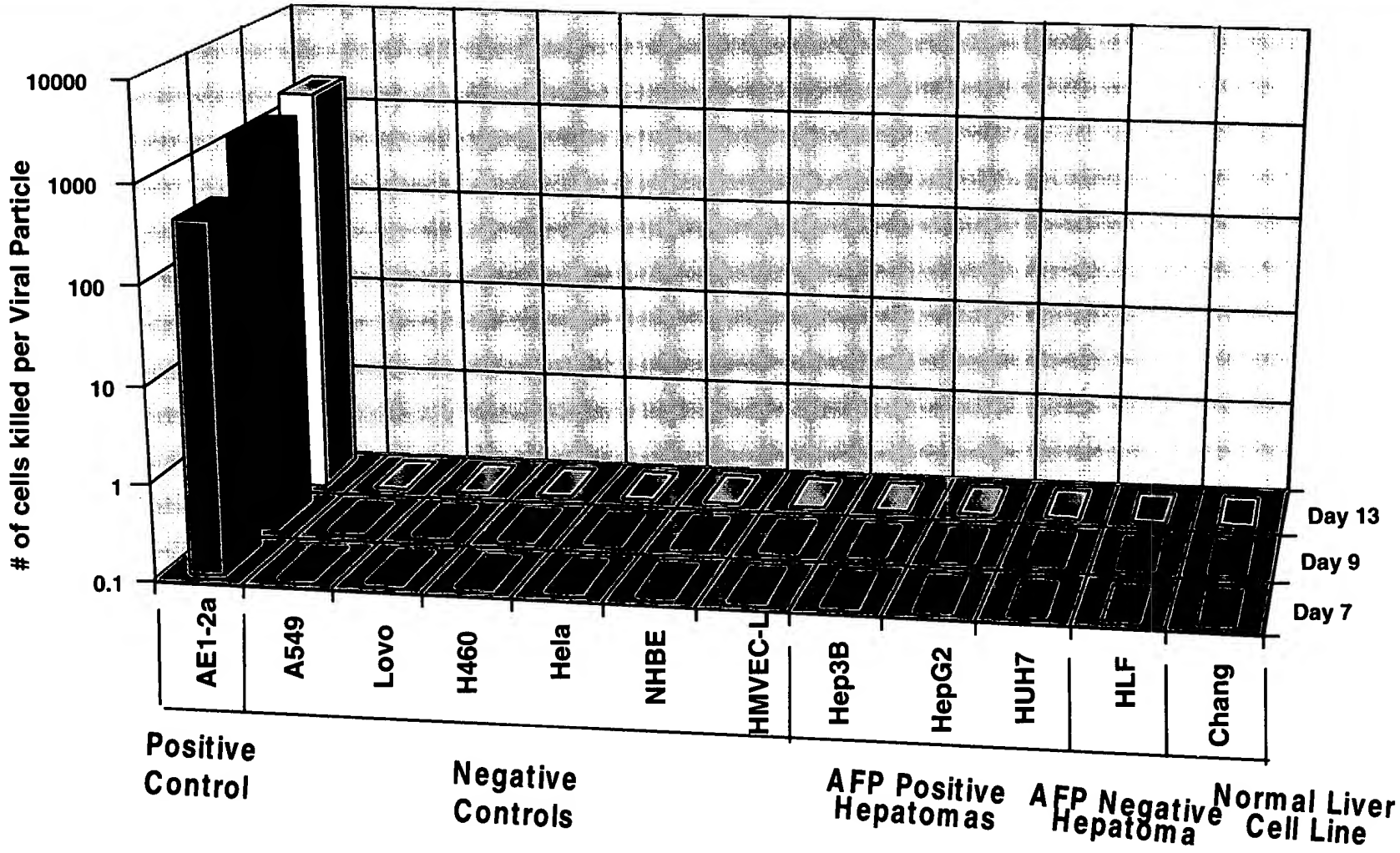
Expression of α-Fetoprotein (AFP) in hepatoma cell lines (Hep3B, HepG2, HuH7, HLF) , non-hepatoma cell lines (AE1-2a, A549, LoVo, H460, HeLa, Chang), and primary human lung cells (NHBE and HMVEC-L). AFP levels of 10 day cell culture supernatant were determined by ELISA (UBI Magiwell Enzyme Immunoassay for α-Fetoprotein , United Biotech, Inc.).

Cell Line Specific Replication of Addl327



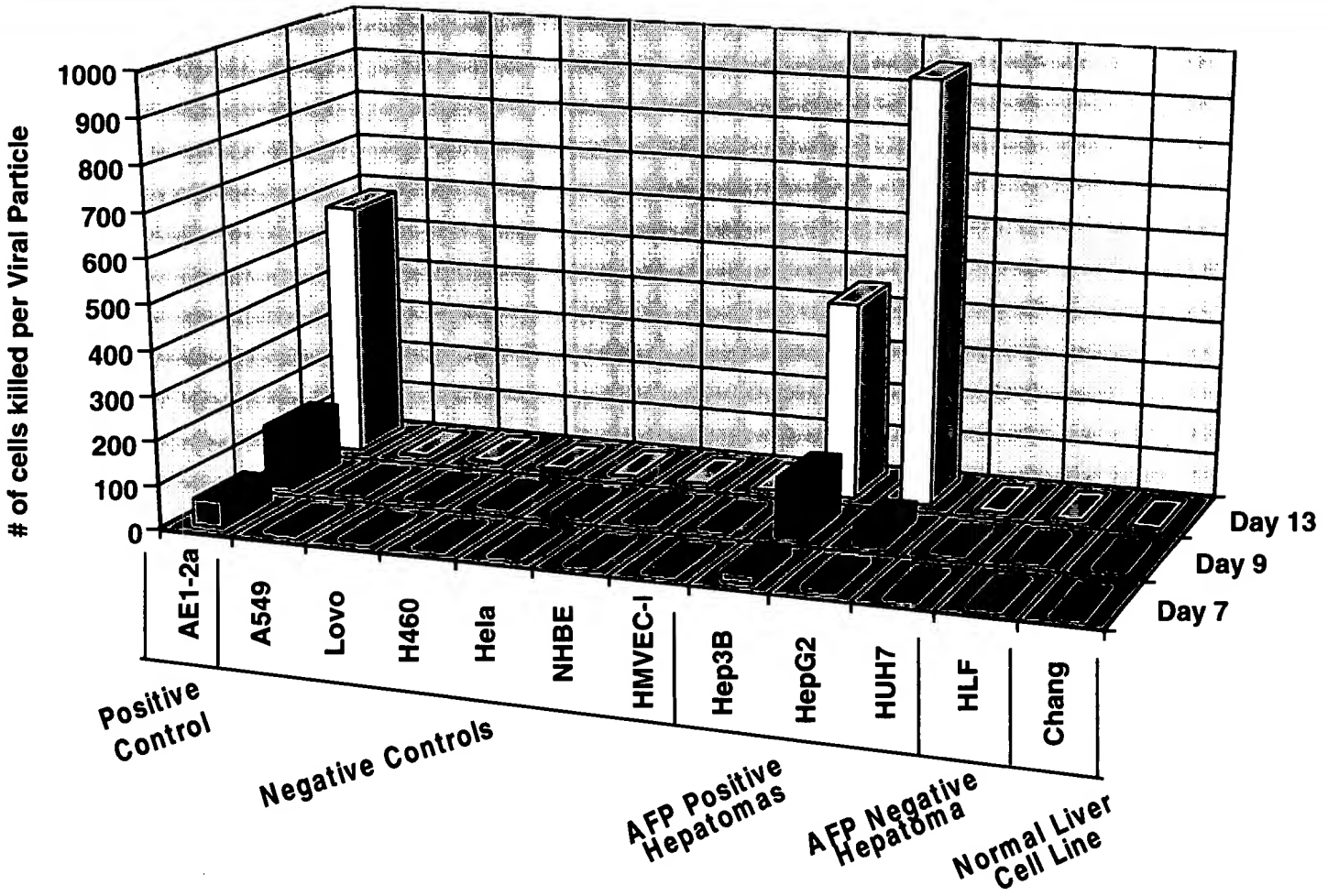
Cytopathic effect of Addl327 (replication competent adenovirus) infection of hepatoma cell lines (Hep3B, HepG2, HuH7, HLF) , non-hepatoma cell lines (AE1-2a, A549, LoVo, H460, HeLa, Chang), and primary human lung cells (NHBE and HMVEC-L). All cell lines used are capable of supporting Addl327 replication and manifesting a cytopathic effect.

Cell Line Specific Replication of Av1nBg01v

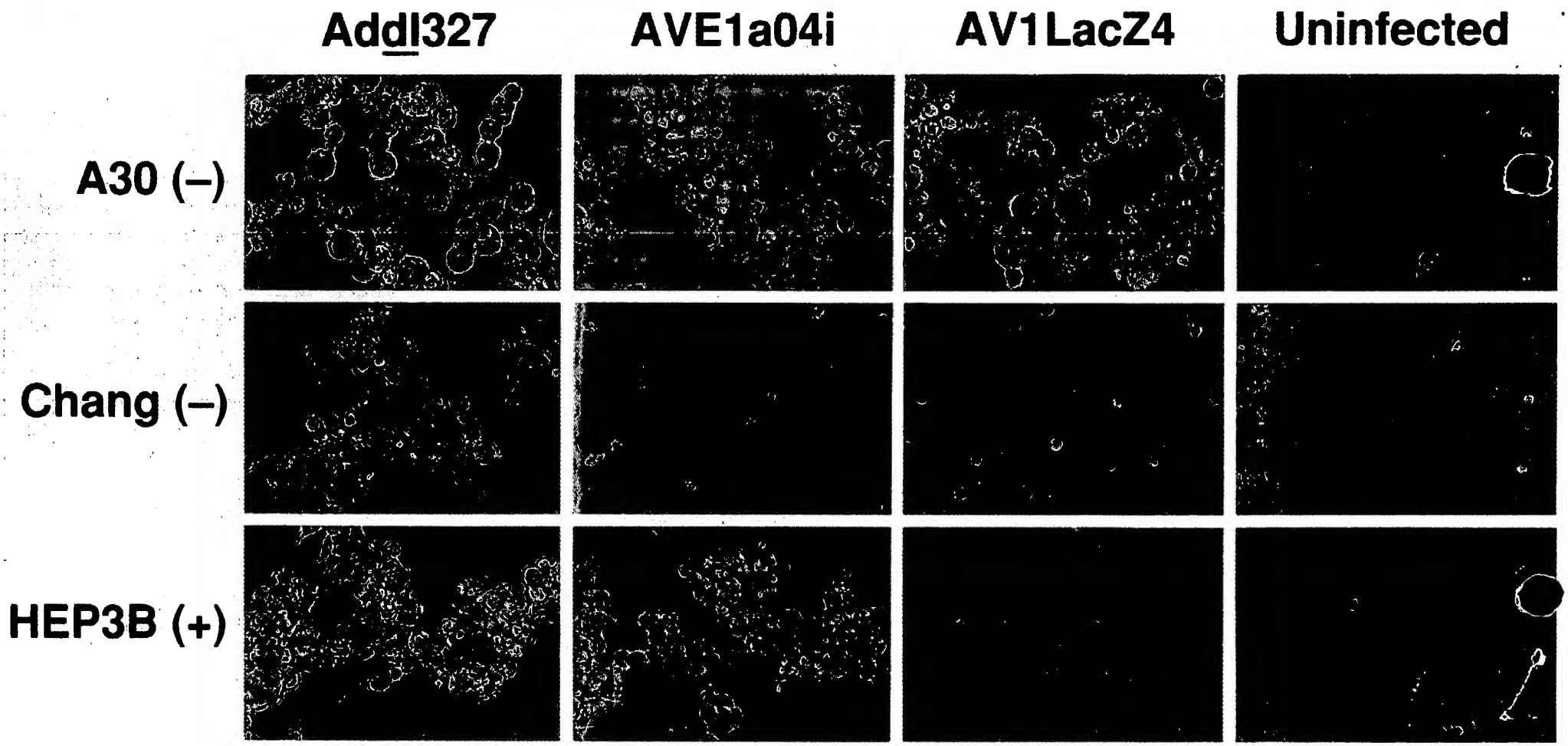


Cytopathic effect of Av1nBg01v (Replication Defective Adenovirus) infection of hepatoma cell lines (Hep3B, HepG2, HuH7, HLF), non-hepatoma cell lines (AE1-2a, A549, LoVo, H460, HeLa, Chang), and primary human lung cells (NHBE and HMVEC-L). Only AE1-2a cells, which provide E1a *in trans*, can support Av1nBg01v replication and manifest a cytopathic effect.

Cell Line Specific Replication of AvE1a04i

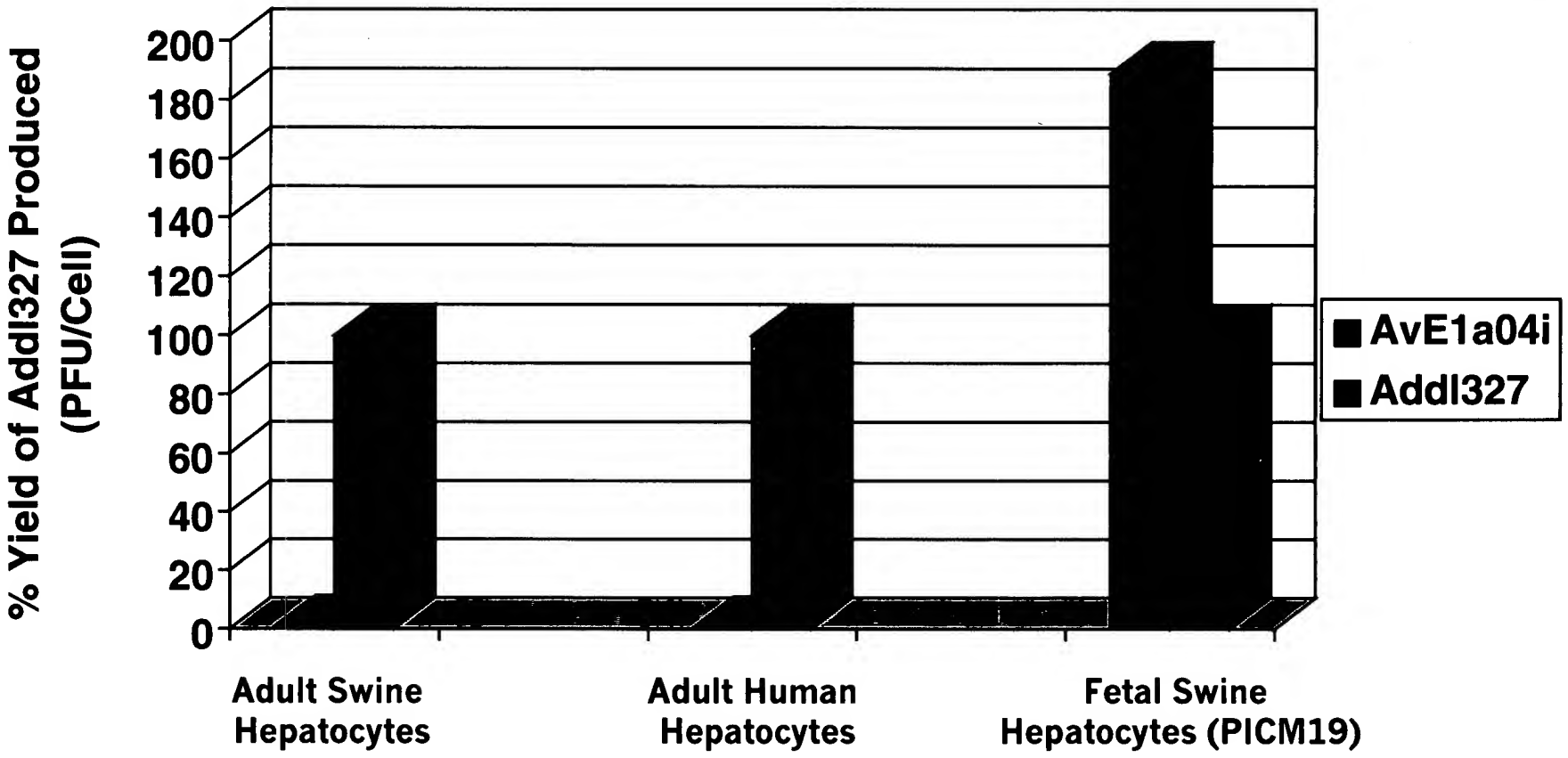


Cytopathic effect of AvE1a04i (Tumor Specific Replication Restricted Adenovirus) infection of hepatoma cell lines (Hep3B, HepG2, HuH7, HLF), non-hepatoma cell lines (AE1-2a, A549, LoVo, H460, HeLa, Chang), and primary human lung cells (NHBE and HMVEC-L). AE1-2a cells, which provide E1a *in trans*, and Hep3B and HepG2, which are AFP^+, can support AvE1a04i replication and manifest a cytopathic effect. All other cell lines, including HuH7 which is also AFP^+, exhibited no cytopathic effect.



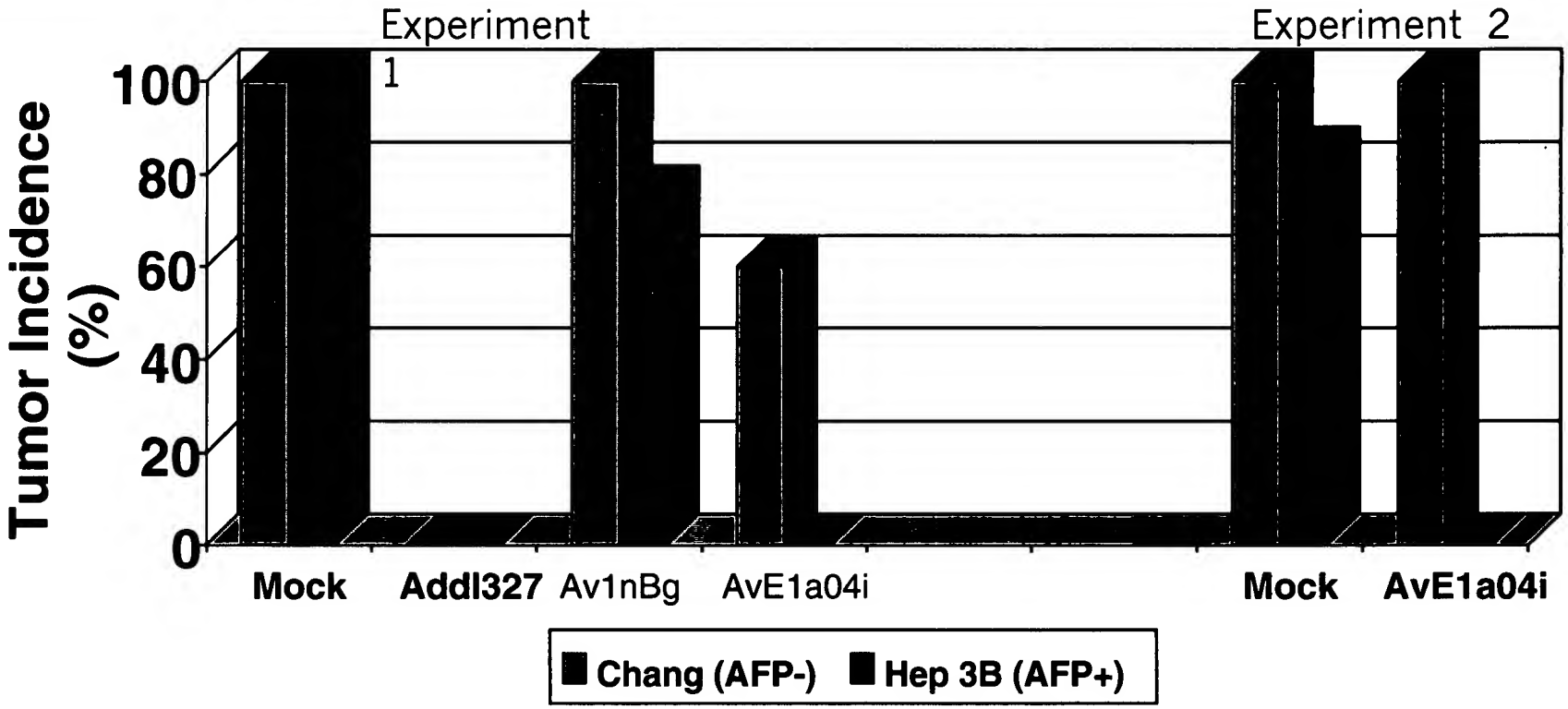
AvE1a04i Specific Replication in Cell Lines. Cytopathic effect of Addl327 (Replication Competent Adenovirus), AvE1a04i (Tumor Specific Replication Restricted Adenovirus), and Av1LacZ4 (Av1nBg01v; Replication Defective Adenovirus) infection of A30 (AE1-2a, AFP- , E1a$^+$ upon dexamethasone induction), Chang (AFP-, non-hepatoma liver cell line), and Hep3B (AFP$^+$, hepatoma cell line).

Specificity of AvE1a04i in Primary Adult Swine and Human Hepatocytes



Replication of AvE1a04i in Primary Hepatocytes. Adult Swine Hepatocytes (AFP^- and cytochrome $P450^+$) and PICM19 cells, an embryonic swine cell line which expresses AFP and has fetal swine hepatocyte characteristics, were generously provided by Dr. Thomas Caperna and Dr. Neil Talbot, respectively, at USDA, Beltsville, MD. Adult Human Hepatocytes were obtained from the Human Cell Culture Center, Inc. Cells were infected at 10 particles per cell with either AvE1a04i or Addl327 and incubated for 7 day. Crude Viral Lysate was prepared and titered by $TCID_{50}$ assay on AE1-2a, which express E1a and can support replication of $E1a^-$ vectors. Titers were then normalized to Addl327 titer and expressed as % yield . These results suggest that swine is a potential immune competent, adenoviral permissive animal model.

Specificity and Efficacy of AvE1a04i in Pre-transduced Xenograft Model-Tumor Incidence



Specificity and Efficiency of AvE1a04i in Pre-transduced Xenographic Model. Experiment 1. Hep3B cells were infected with either 50 particles per cell of Addl327, Av1nBg01v, or AvE1a04i, or were uninfected, incubated for 16 hour, and then mixed with uninfected Hep3B cells at a ratio of 10:1 uninfected to infected. A total of 1 x 10^7 cells were then implanted subcutaneously into the right flank of BINX (beige, nude, XID) immunodeficient mice. Tumor growth was monitored by caliper measurement. Chang cells were infected at 500 particles per cell and otherwise handled identically. *In vitro* studies with Av1nBg demonstrated 100% infection at these MOIs. Experiment 2. Hep3B cells were infected with 2 particles per cell of AvE1a04i or were uninfected for 16 hours, then 1 x 10^7 cells were implanted and monitored as before. Chang cells were infected with 10 particles per cell and otherwise handled identically. *In vitro* studies with Av1nBg demonstrated 1% infection at these MOIs.

Conclusion:

1. **As few as 1 % of Hep 3B cells need to be infected with AvE1a04i to prevent tumor growth.**
2. **Little effect on Chang cell tumors when 1 % of cells transduced with AvE1a04i.**

Efficacy of AvE1a04i in Pre-Established Xenographic Tumor